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(71) Applicant: CENTOCOR [US/US]; 200 GREAT VALLEY PARKWAY, MALVERN, PA 19355 (US).

(72) Inventor: SCALLON, Bernard; 139 HEMLOCK DRIVE, COLLEGEVILLE, PA 19426 (US).

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(54) Title: RSV PROTEINS, ANTIBODIES, COMPOSITIONS, METHODS AND USES

HC -

HCH1

EVQLLEESGGGLVRLPGSLRLSCAASGTTLSGYTMHWVRQAPGKGLEWVSSITGGSNFINYSDSVKG  
RFTISRDNKNSLYLQMNSLTAEFTAVYYCATAPIAPPYFDHWGQGLTVTVSS

HCH2

EVQLLEESGGGLVRLPGSLRLSCAASGTTLSGYTMHWVRQAPGKGLEWVSSITGGSNFINYSDSVKGR  
FTISRDNKNSLYLQMNSLTAEFTAVYYCATAPIAPPYFDHWGQGLTVTVSS

LC -

L1

DIQMTQSPSSLSASVGDRVTITCRATQSVSNFLNHWYQQKPGEAPTLTIYDASTSQSGVPSRFSGSGS  
GMDFSLTISSLQPEDLAMYYCQASINTPLFGGGTRIDMR

L2

DIQMTQSPSSLSASVGDRVTITCRATQSVSNFLNHWYQQKPGEAPTLTIYDASTSQSGVPSRFSGSGS  
GMDFSLTISSLQPEDLAMYYCQASINTPLFGGGTRVDIK

L3

DAELTQSPSSLSASVGDRVTITCRATQSVSNFLNHWYQQKPGEAPTLTIYDASTSQSGVPSRFSGSGS  
GMDFSLTISSLQPEDLAMYYCQASINTPLFGGGTRIDMR

L4

AELTQSPSSLSASVGDRVTITCRATQSVSNFLNHWYQQKPGEAPTLTIYDASTSQSGVPSR  
FSGSGSGMDFSLTISSLQPEDLAMYYCQASINTPLFGGGTRIDMR

(57) Abstract: The present invention relates to at least one novel RSV proteins, antibodies, including isolated nucleic acids that encode at least one RSV protein or antibody, RSV vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.

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## RSV PROTEINS, ANTIBODIES, COMPOSITIONS, METHODS AND USES

5

### BACKGROUND OF THE INVENTION

#### FIELD OF THE INVENTION

The present invention relates to at least one respiratory syncytial virus (RSV) protein or  
10 fragment thereof, and antibodies, including specified portions or variants, specific therefore, as well  
as nucleic acids encoding such RSV proteins, fragments, antibodies, complementary nucleic acids,  
vectors, host cells, and methods of making and using thereof, including therapeutic formulations,  
administration and devices.

#### 15 RELATED ART

Respiratory syncytial virus (RSV) is a Paramyxovirus of the Pneumovirus genus which  
commonly infects the upper and lower respiratory tract. It is so contagious that by age two, a large  
percentage of children have been infected by it. Moreover, by age four, virtually all humans have an  
immunity to RSV. Typically, RSV infections are mild, remaining localized in the upper respiratory  
20 tract and causing symptoms similar to a common cold which require no extensive treatment.  
However, in some subjects, e.g., immunosuppressed individuals such as infants, elderly persons or  
patients with underlying cardiopulmonary diseases, the virus may penetrate to the lower respiratory  
tract requiring hospitalization and breathing support. In some of these cases, RSV infection may  
cause permanent lung damage or even be life threatening. In the United States alone, RSV results in  
25 about 90,000 hospitalizations each year, and results in about 4500 deaths.

RSV appears in two major strain subgroups, A and B, primarily based on serological  
differences associated with the attachment glycoprotein, G. The major surface glycoprotein, i.e., the  
90 kD G protein, can differ up to 50% at the amino acid level between isolates. By contrast, a  
potential therapeutic target, the 70 kD fusion (F) protein, is highly conserved across different RSV  
30 strains, about i.e., 89% on the amino acid level. Moreover, it is known that antibodies elicited  
against F-protein of a given type are cross-reactive with the other type.

The F-protein is a heterodimer, generated from a linear precursor, consisting of disulfide-  
linked fragments of 48 and 23 kD respectively. Inhibition of syncytia formation by polyclonal

antibodies is associated with significant reaction to the 23 kD fragment. As noted, while RSV infections are usually mild, in some individuals RSV infections may be life threatening.

However, notwithstanding the previous published reports of humanized and Fab fragments specific to RSV, there still exists a significant need for improved anti-RSV antibodies having improved therapeutic potential, in particular anti-RSV antibodies which possess high affinity and specificity for the RSV F-protein which effectively neutralize and prevent RSV infection.

Non-human mammalian, chimeric, polyclonal (e.g., sera) and/or monoclonal antibodies (Mabs) and fragments (e.g., proteolytic digestion or fusion protein products thereof) are potential therapeutic agents that are being investigated in some cases to attempt to treat certain diseases.

However, such antibodies or fragments can elicit an immune response when administered to humans. Such an immune response can result in an immune complex-mediated clearance of the antibodies or fragments from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the readministration of the antibody or fragment. For example, repeated administration of antibodies or fragments comprising non-human portions can lead to serum sickness and/or anaphalaxis. In order to avoid these and other problems, a number of approaches have been taken to reduce the immunogenicity of such antibodies and portions thereof, including chimerization and humanization, as well known in the art. These and other approaches, however, still can result in antibodies or fragments having some immunogenicity, low affinity, low avidity, or with problems in cell culture, scale up, production, and/or low yields. Thus, such antibodies or fragments can be less than ideally suited for manufacture or use as therapeutic proteins.

Accordingly, there is a need to provide RSV proteins or antibodies or fragments that overcome one more of these problems, as well as improvements over known proteins or antibodies or fragments thereof.

## **SUMMARY OF THE INVENTION**

The present invention provides isolated human, primate, rodent, mammalian, chimeric, or human RSV proteins, antibodies, immunoglobulins, cleavage products and other specified portions and variants thereof, as well as RSV protein or antibody compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods of making and using thereof, as described and enabled herein, in combination with what is known in the art.

The present invention also provides at least one isolated RSV antibody as described herein. An antibody according to the present invention can include any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) (also termed the hypervariable region or HV) of a heavy or light chain variable region, or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, wherein the antibody can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, or any combination thereof, and the like.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding specific RSV proteins or antibodies, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising at least one of said RSV protein or antibody encoding or complementary nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such antibody nucleic acids, vectors and/or host cells.

At least one antibody of the invention binds at least one specified epitope specific to at least one RSV protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least 1-5 amino acids of at least one portion thereof, such as but not limited to, at least one functional, extracellular, soluble, hydrophilic, external or cytoplasmic domain of said protein, or any portion thereof.

The at least one antibody can optionally comprise at least one specified portion of at least one complementarity determining region (CDR) (e.g., CDR1, CDR2 or CDR3 of the heavy or light chain variable region) and optionally at least one constant or variable framework region or any portion thereof. The at least one antibody amino acid sequence can further optionally comprise at least one specified substitution, insertion or deletion as described herein or as known in the art.

The present invention also provides at least one isolated RSV protein or antibody as described herein, wherein the antibody has at least one activity, such as, but not limited to any known RSV biological activity. A(n) RSV protein antibody can thus be screened for a corresponding activity according to known methods, such as but not limited to, at least one biological activity towards a RSV protein or protein related function.



The present invention further provides at least one RSV anti-idiotypic antibody to at least one RSV antibody of the present invention. The anti-idiotypic antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, and the like. The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding at least one RSV anti-idiotypic antibody, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said RSV anti-idiotypic antibody encoding nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such anti-idiotypic antibody nucleic acids, vectors and/or host cells.

The present invention also provides at least one method for expressing at least one RSV protein or antibody, or RSV anti-idiotypic antibody, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one RSV antibody is expressed in detectable and/or recoverable amounts.

The present invention also provides at least one composition comprising (a) an isolated RSV protein or antibody encoding nucleic acid and/or protein or antibody as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, such as but not limited to known carriers or diluents. The composition can optionally further comprise at least one further compound, protein or composition.

The present invention further provides at least one RSV protein or antibody method or composition, for administering a therapeutically effective amount to modulate or treat at least one RSV related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one RSV protein or antibody, according to the present invention.

The present invention further provides at least one RSV protein or antibody method or composition, for diagnosing at least one RSV related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as

described herein.

The present invention also provides at least one composition, device and/or method of delivery for diagnosing of at least one RSV protein or antibody, according to the present invention.

Also provided is an isolated nucleic acid encoding at least one isolated mammalian RSV protein; an isolated nucleic acid vector comprising the isolated nucleic acid, and/or a prokaryotic or eukaryotic host cell comprising the isolated nucleic acid. The host cell can optionally be at least one selected from prokaryotic or eukaryotic cells, or fusion cells thereof, e.g., but not limited to, mammalian, plant or insect, such as but not limited to, CHO, myeloma, or lymphoma cells, bacterial cells, yeast cells, silk worm cells, or any derivative, immortalized or transformed cell thereof. Also provided is a method for producing at least one RSV protein, comprising translating the protein encoding nucleic acid under conditions in vitro, in vivo or in situ, such that the RSV protein is expressed in detectable or recoverable amounts.

Also provided is a composition comprising at least one isolated mammalian RSV protein and at least one pharmaceutically acceptable carrier or diluent. The composition can optionally further comprise an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

Also provided is a method for diagnosing or treating a RSV related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one isolated mammalian RSV protein of the invention with, or to, the cell, tissue, organ or animal. The method can optionally further comprise using an effective amount of 0.0000001-500 mg/kilogram of the cells, tissue, organ or animal. The method can optionally further comprise using the contacting or the administering by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal,

intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal. The method can optionally further comprise administering, prior, concurrently or after the contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, an anti-inflammatory, a non-steroid inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

Also provided is at least one medical device, comprising at least one isolated mammalian RSV protein of the invention, wherein the device is suitable to contacting or administering the at least one RSV protein by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelular, intracerebellar, intracerebroventricular, intracolonic, intracervical, intragastric, intrahepatic, intramyocardial, intraosseous, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

Also provided is an article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one isolated mammalian RSV protein of the present invention. The article of manufacture can optionally comprise having the container as a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelular, intracerebellar, intracerebroventricular, intracolonic, intracervical, intragastric, intrahepatic, intramyocardial, intraosseous, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

Also provided is a method for producing at least one isolated mammalian RSV protein of the present invention, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts the protein. Further provided in the present

invention is at least one RSV protein produced by the above method.

In another aspect, the present invention provides at least one isolated mammalian RSV antibody, comprising at least one variable region comprising an amino acid sequence of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F .

5 In another aspect, the present invention provides at least one isolated mammalian RSV antibody, comprising all of the heavy chain or all of the light chain complementarity determining regions (CDR) amino acid sequences comprising an amino acid sequence of SEQ ID NOS:1-6, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F .

10 In another aspect, the present invention provides at least one isolated mammalian RSV antibody, comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least part of at least one of SEQ ID NOS: 1-6.

In other aspect the present invention provides at least one isolated mammalian RSV antibody, comprising at least one human CDR, wherein the antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of any know RSV protein, such as the F glycoprotein.

15 The at least one antibody can optionally further at least one of: bind RSV with an affinity of at least one selected from at least  $10^{-9}$  M, at least  $10^{-10}$  M, at least  $10^{-11}$  M, or at least  $10^{-12}$  M; substantially neutralizes at least one activity of at least one RSV protein. Also provided is an isolated nucleic acid encoding at least one isolated mammalian RSV antibody; an isolated nucleic acid vector  
20 comprising the isolated nucleic acid, and/or a prokaryotic or eukaryotic host cell comprising the isolated nucleic acid. The host cell can optionally be at least one selected from prokaryotic or eukaryotic cells, or fusion cells thereof, e.g., but not limited to, mammalian, plant or insect, such as but not limited to, CHO, myeloma, or lymphoma cells, bacterial cells, yeast cells, silk worm cells, or any derivative, immortalized or transformed cell thereof. Also provided is a method for producing at  
25 least one RSV antibody, comprising translating the antibody encoding nucleic acid under conditions in vitro, in vivo or in situ, such that the RSV antibody is expressed in detectable or recoverable amounts.

Also provided is a composition comprising at least one isolated mammalian RSV antibody and at least one pharmaceutically acceptable carrier or diluent. The composition can optionally  
30 further comprise an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a



neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a  
5 cytokine antagonist.

The present invention further provides an anti-idiotypic antibody or fragment that specifically binds at least one isolated mammalian RSV antibody of the present invention.

Also provided is a method for diagnosing or treating a RSV related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective  
10 amount of at least one isolated mammalian RSV antibody of the invention with, or to, the cell, tissue, organ or animal. The method can optionally further comprise using an effective amount of 0.0001-500 mg/kilogram of the cells, tissue, organ or animal. The method can optionally further comprise using the contacting or the administering by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular,  
15 intracartilaginous, intracavitary, intracellic, intracerebellar, intracerebroventricular, intracolonic, intracervical, intragastric, intrahepatic, intramyocardial, intraosseous, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal. The method can optionally further comprise administering,  
20 prior, concurrently or after the contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, an anti-inflammatory, a non-steroid inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an  
25 erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

Also provided is at least one medical device, comprising at least one isolated mammalian  
30 RSV antibody of the invention, wherein the device is suitable to contacting or administering the at least one RSV antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracellic, intracerebellar, intracerebroventricular, intracolonic, intracervical, intragastric,

intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

5 Also provided is an article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one isolated mammalian RSV antibody of the present invention. The article of manufacture can optionally comprise having the container as a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular,  
10 intracartilaginous, intracavitary, intracelial, intracebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

15 Also provided is a method for producing at least one isolated mammalian RSV antibody of the present invention, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts the antibody. Further provided in the present invention is at least one RSV antibody produced by the above method.

The present invention further provides any invention described herein.

## 20 DESCRIPTION OF THE FIGURES

Figure 1 shows two heavy chain and three light chain antibody amino acid sequences of the present invention.

25 Figure 2A-G shows a non-limiting example of two heavy chain region encoding DNA sequences encoding an RSV antibody of the present invention.

Figure 3 shows a non-limiting example of two heavy chain amino acid sequences corresponding to the DNA sequences in Figure 2A-G, where CDR1, 2 &3, variable and constant regions and a signal peptide are shown, as well as variations between the alternative sequences are shown.

30 Figure 4 shows a non-limiting example of four different light chain amino acid sequences that can be used in an RSV antibody of the present invention, where CDR1, 2 &3, variable and constant regions and a signal peptide are shown, as well as variations between the alternative

sequences are shown.

Figure 5A-F shows a non-limiting example of four alternative light chain region encoding DNA sequences encoding an RSV antibody of the present invention, corresponding to the amino acid sequence in Figure 4.

## 5 DESCRIPTION OF THE INVENTION

The present invention provides isolated, recombinant and/or synthetic RSV human, primate, rodent, mammalian, chimeric, humanized or CDR-grafted, antibodies and RSV anti-idiotypic antibodies thereto, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one RSV antibody or anti-idiotypic antibody. The present invention further includes, but is not limited to, methods of making and using such nucleic acids and antibodies and anti-idiotypic antibodies, including diagnostic and therapeutic compositions, methods and devices.

As used herein, an "respiratory syncytial virus antibody," "RSV antibody," and the like include any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion, fragment or variant thereof, or at least one portion of an RSV receptor or binding protein, which can be incorporated into a RSV antibody of the present invention.

Antibodies can include one or more of at least one CDR, at least one variable region, at least one constant region, at least one heavy chain (e.g.,  $\gamma_1$ ,  $\gamma_2$ ,  $\gamma_3$ ,  $\gamma_4$ ,  $\mu$ ,  $\alpha_1$ ,  $\alpha_2$ ,  $\delta$ ,  $\epsilon$ ), at least one light chain (e.g.,  $\kappa$  and  $\lambda$ ), or any portion or fragment thereof, and can further comprise interchain and intrachain disulfide bonds, hinge regions, glycosylation sites that can be separated by a hinge region, as well as heavy chains and light chains. Light chains typically have a molecular weight of about 25Kd and heavy chains typically range from 50K-77Kd. Light chains can exist in two distinct forms or isotypes, kappa ( $\kappa$ ) and lambda ( $\lambda$ ), which can combine with any of the heavy chain types. All light chains have at least one variable region and at least one constant region. The IgG antibody is considered a typical antibody structure and has two intrachain disulfide bonds in the light chain (one in variable region and one in the constant region), with four in the heavy chain, and such bond encompassing a peptide loop of about 60-70 amino acids comprising a "domain" of about 110 amino acids in the chain. IgG antibodies can be characterized into four classes, IgG1, IgG2, IgG3 and IgG4.

Each immunoglobulin class has a different set of functions. The following table summarizes the Physicochemical properties of each of the immunoglobuling classes and subclasses.

Property	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	SIgA	IgD	IgE
Heavy Chain	$\gamma 1$	$\gamma 1$	$\gamma 1$	$\gamma 1$	$\mu$	$\alpha 1$	$\alpha 2$	$\alpha 1 / \alpha 2$	$\delta$	e
Mean Serum conc. (mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.05	0.03	0.00005
Sedimentation constant	7s	7s	7s	7s	19s	7s	7s	11s	7s	8s
Mol. Wt. ( $\times 10^3$ )	146	146	170	146	970	160	160	385	184	188
Half Life (days)	21	20	7	21	10	6	6	?	3	2
% intravascular distribution	45	45	45	45	80	42	42	Trac e	75	50
Carbohydrate (%)	2-3	2-3	2-3	2-3	12	7-11	7-11	7-11	9-14	12

The following table summarizes non-limiting examples of antibody effector functions for

5 human antibody classes and subclasses.

Effector function	IgG1	IgG2	IgG3	IgG4	IgM	IgA	IgD	IgE
Complement fixation	++	+	+++	--	+++	--	--	-
Placental transfer	+	+	+	+	-	-	-	-
Binding to Staph A	+++	+++	-	+++	-	-	-	-
Binding to Strep G	+++	+++	+++	+++	-	-	-	-

Accordingly, the type of antibody or fragment thereof can be selected for use according to the present invention based on the desired characteristics and functions that are desired for a particular therapeutic or diagnostic use, such as but not limited to serum half life, intravascular distribution, complement fixation, etc.

Antibody diversity is generated by at least 5 mechanisms, including (1) the use of multiple genes encoding parts of the antibody; (2) somatic mutation, e.g., primordial V gene mutation during B-cell ontogeny to produce different V genes in different B-cell clones; (3) somatic recombination, e.g., gene segments J1-Jn recombine to join the main part of the V-region gene during B-cell ontogeny; (4) gene conversion where sections of DNA from a number of pseudo V region can be copied into the V region to alter the DNA sequence; and (5) nucleotide addition, e.g., when V and J regions are cut, before joining, and extra nucleotides may be inserted to code for additional amino acids. Non-limiting examples include, but are not limited to, (i) the selection/recombination of V $\kappa$ , J, and C $\kappa$  regions from germ line to B-cell clones to generate kappa chains; (ii) selection/recombination of V $\lambda$ , J, and C $\lambda$  regions from germ line to B-cell clones to generate lambda chains; (iii) selection/recombination of V $_H$ , D1-D30 and J $_H$ 1-J $_H$ 6 genes to form a functional VDJ gene encoding a



heavy chain variable region. The above mechanisms work in a coordinated fashion to generate antibody diversity and specificity.

The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a mammalian RSV. For example, antibody fragments capable of binding to RSV or portions thereof, including, but not limited to Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and F(ab')<sub>2</sub> (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, supra).

Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a F(ab')<sub>2</sub> heavy chain portion can be designed to include DNA sequences encoding the CH<sub>1</sub> domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C<sub>L</sub>, C<sub>H</sub> domains (e.g., C<sub>H</sub>1, C<sub>H</sub>2, C<sub>H</sub>3), hinge, (V<sub>L</sub>, V<sub>H</sub>)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such

as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

5 Bispecific, heterospecific, heteroconjugate or similar antibodies can also be used that are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for at least one RSV protein, the other one is for any other antigen. Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have  
10 different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed, e.g., in  
15 WO 93/08829, US Patent Nos, 6210668, 6193967, 6132992, 6106833, 6060285, 6037453, 6010902, 5989530, 5959084, 5959083, 5932448, 5833985, 5821333, 5807706, 5643759, 5601819, 5582996, 5496549, 4676980, WO 91/00360, WO 92/00373, EP 03089, Traunecker et al., EMBO J. 10:3655 (1991), Suresh et al., Methods in Enzymology 121:210 (1986), each entirely incorporated herein by reference.

20 Such antibodies optionally further affect a specific ligand, such as but not limited to where such antibody modulates, decreases, increases, antagonizes, antagonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with at least one RSV activity or binding, or with RSV receptor activity or binding, *in vitro*, *in situ* and/or *in vivo*. As a non-limiting example, a suitable RSV antibody, specified portion or variant of the present invention can bind at least one RSV, or specified  
25 portions, variants or domains thereof. A suitable RSV antibody, specified portion, or variant can also optionally affect at least one of RSV activity or function, such as but not limited to, RNA, DNA or protein synthesis, RSV release, RSV receptor signaling, membrane RSV cleavage, RSV activity, RSV production and/or synthesis.

30 RSV antibodies (also termed RSV antibodies) useful in the methods and compositions of the present invention can optionally be characterized by high affinity binding to RSV and optionally and preferably having low toxicity. In particular, an antibody, specified fragment or variant of the invention, where the individual components, such as the variable region, constant region and framework, individually and/or collectively, optionally and preferably possess low immunogenicity,

is useful in the present invention. The antibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with measurable alleviation of symptoms and low and/or acceptable toxicity. Low or acceptable immunogenicity and/or high affinity, as well as other suitable properties, can contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAHA, HACA or HAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (Elliott *et al.*, *Lancet* 344:1125-1127 (1994), entirely incorporated herein by reference).

#### 10 Utility

The isolated nucleic acids of the present invention can be used for production of at least one RSV antibody or specified variant thereof, which can be used to measure or effect in an cell, tissue, organ or animal (including mammals and humans), to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one RSV condition, selected from, but not limited to, at least one of an immune disorder or disease, a cardiovascular disorder or disease, an infectious, malignant, and/or neurologic disorder or disease, or other known or specified RSV related condition.

Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one RSV antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.001 to 500 mg/kg per single (e.g., bolus), multiple or continuous administration, or to achieve a serum concentration of 0.01-5000 µg/ml serum concentration per single, multiple, or continuous administration, or any effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts.

#### 25 Citations

All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats.

The following references are entirely incorporated herein by reference: Ausubel, et al., ed., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY (1989); Harlow and

Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001).

#### Antibodies of the Present Invention

5           At least one RSV antibody of the present invention can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring  
10 Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), each entirely incorporated herein by reference.

          Human antibodies that are specific for human RSV proteins or fragments thereof can be raised against an appropriate immunogenic antigen, such as isolated and/or RSV protein or a portion  
15 thereof (including synthetic molecules, such as synthetic peptides). Other specific or general mammalian antibodies can be similarly raised. Preparation of immunogenic antigens, and monoclonal antibody production can be performed using any suitable technique.

          In one approach, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243,  
20 P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art. See, e.g., [www.atcc.org](http://www.atcc.org), [www.lifetech.com](http://www.lifetech.com), and the like, with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood,  
25 lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA,  
30 single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, entirely incorporated herein by reference.



Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Biovation, Aberdeen, Scotland, UK; BioInvent, Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, CA; Ixsys. See, e.g., EP 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT/GB92/00883; PCT/GB93/00605; US 08/350260(5/12/94); PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC); WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scripps); EP 614 989 (MorphoSys); WO95/16027 (BioInvent); WO88/06630; WO90/3809 (Dyax); US 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 550 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated peptides or proteins - US 5723323, 5763192, 5814476, 5817483, 5824514, 5976862, WO 86/05803, EP 590 689 (Ixsys, now Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., Microbiol. Immunol. 41:901-907 (1997); Sandhu et al., Crit. Rev. Biotechnol. 16:95-118 (1996); Eren et al., Immunol. 93:154-161 (1998), each entirely incorporated by reference as well as related patents and applications) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al., Proc. Natl. Acad. Sci. USA, 94:4937-4942 (May 1997); Hanes et al., Proc. Natl. Acad. Sci. USA, 95:14130-14135 (Nov. 1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (US pat. No. 5,627,052, Wen et al., J. Immunol. 17:887-892 (1987); Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843-7848 (1996)); gel microdroplet and flow cytometry (Powell et al., Biotechnol. 8:333-337 (1990); One Cell Systems, Cambridge, MA; Gray et al., J. Imm. Meth. 182:155-163 (1995); Kenny et al., Bio/Technol. 13:787-790 (1995)); B-cell

selection (Steenbakkers et al., Molec. Biol. Reports 19:125-134 (1994); Jonak et al., Progress Biotech, Vol. 5, In Vitro Immunization in Hybridoma Technology, Borrebaeck, ed., Elsevier Science Publishers B.V., Amsterdam, Netherlands (1988)).

Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. Generally, a humanized or engineered antibody has one or more amino acid residues from a source which is non-human, e.g., but not limited to mouse, rat, rabbit, non-human primate or other mammal. These human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable, constant or other domain of a known human sequence. Known human Ig sequences are disclosed, e.g.,

www.ncbi.nlm.nih.gov/entrez/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/;  
www.abcam.com/; www.antibodyresource.com/onlinecomp.html;  
www.public.iastate.edu/~pedro/research\_tools.html; www.mgen.uni-heidelberg.de/SD/TT/TT.html;  
www.whfreeman.com/immunology/CH05/kuby05.htm;  
www.library.thinkquest.org/12429/Immune/Antibody.html;  
www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/~mrc7/mikeimages.html;  
www.antibodyresource.com/;  
mcb.harvard.edu/BioLinks/Immunology.html.www.immunologylink.com/;  
pathbox.wustl.edu/~hcenter/index.html; www.biotech.ufl.edu/~hcl/;  
www.pebio.com/pa/340913/340913.html; www.nal.usda.gov/awic/pubs/antibody/;  
www.m.ehime-u.ac.jp/~yasuhito/Elisa.html; www.biodesign.com/table.asp;  
www.icnet.uk/axp/facs/davies/links.html; www.biotech.ufl.edu/~fccl/protocol.html; www.isac-net.org/sites\_geo.html; aximt1.imt.uni-marburg.de/~rek/AEPStart.html;  
baserv.uci.kun.nl/~jraats/links1.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V\_mice.html; imgt.cnusc.fr:8104/;  
www.biochem.ucl.ac.uk/~martin/abs/index.html; antibody.bath.ac.uk/;  
abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/~honegger/AHOseminar/Slide01.html;  
www.cryst.bbk.ac.uk/~ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm;  
www.path.cam.ac.uk/~mrc7/humanisation/TAHHP.html;  
www.ibt.unam.mx/vir/structure/stat\_aim.html; www.biosci.missouri.edu/smithgp/index.html;  
www.cryst.bioc.cam.ac.uk/~fmolina/Web-pages/Pept/spottech.html; www.jerini.de/fr\_products.htm;  
www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference.

Such imported sequences can be used to reduce immunogenicity or reduce, enhance or

modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids. antibodies can also optionally be humanized with retention of high

5 affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-

10 dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity

15 for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in, Winter (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol.

20 Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), US patent Nos: 5723323, 5976862, 5824514, 5817483, 5814476, 5763192, 5723323, 5,766886, 5714352, 6204023, 6180370, 5693762, 5530101, 5585089, 5225539; 4816567, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, each entirely

25 incorporated herein by reference, included references cited therein.

The RSV antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human RSV antibody can be isolated from such animals and immortalized using suitable methods, such as the

30 methods described herein.

Transgenic mice that can produce a repertoire of human antibodies that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos: 5,770,428, 5,569,825, 5,545,806, 5,625,126, 5,625,825, 5,633,425, 5,661,016 and 5,789,650 issued to Lonberg *et*

*al.*; Jakobovits *et al.* WO 98/50433, Jakobovits *et al.* WO 98/24893, Lonberg *et al.* WO 98/24884, Lonberg *et al.* WO 97/13852, Lonberg *et al.* WO 94/25585, Kucherlapate *et al.* WO 96/34096, Kucherlapate *et al.* EP 0463 151 B1, Kucherlapate *et al.* EP 0710 719 A1, Surani *et al.* US. Pat. No. 5,545,807, Bruggemann *et al.* WO 90/04036, Bruggemann *et al.* EP 0438 474 B1, Lonberg *et al.* EP 0814 259 A2, Lonberg *et al.* GB 2 272 440 A, Lonberg *et al.* *Nature* 368:856-859 (1994), Taylor *et al.*, *Int. Immunol.* 6(4):579-591 (1994), Green *et al.*, *Nature Genetics* 7:13-21 (1994), Mendez *et al.*, *Nature Genetics* 15:146-156 (1997), Taylor *et al.*, *Nucleic Acids Research* 20(23):6287-6295 (1992), Tuailon *et al.*, *Proc Natl Acad Sci USA* 90(8):3720-3724 (1993), Lonberg *et al.*, *Int Rev Immunol* 13(1):65-93 (1995) and Fishwald *et al.*, *Nat Biotechnol* 14(7):845-851 (1996), which are each entirely incorporated herein by reference). Generally, these mice comprise at least one transgene comprising DNA from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes.

Screening antibodies for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, CA), and Cambridge antibody Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4704692, 4939666, 4946778, 5260203, 5455030, 5518889, 5534621, 5656730, 5763733, 5767260, 5856456, assigned to Enzon; 5223409, 5403484, 5571698, 5837500, assigned to Dyax, 5427908, 5580717, assigned to Affymax; 5885793, assigned to Cambridge antibody Technologies; 5750373, assigned to Genentech, 5618920, 5595898, 5576195, 5698435, 5693493,



5698417, assigned to Xoma, Colligan, *supra*; Ausubel, *supra*; or Sambrook, *supra*, each of the above patents and publications entirely incorporated herein by reference.

Antibodies of the present invention can also be prepared using at least one RSV antibody encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, US patent nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Antibodies of the present invention can additionally be prepared using at least one RSV antibody encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., Curr. Top. Microbol. Immunol. 240:95-118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., Adv. Exp. Med. Biol. 464:127-147 (1999) and references cited therein. antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., Plant Mol. Biol. 38:101-109 (1998) and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to know methods. See also, e.g., Fischer et al., Biotechnol. Appl. Biochem. 30:99-108 (Oct., 1999), Ma et al., Trends Biotechnol. 13:522-7 (1995); Ma et al., Plant Physiol. 109:341-6 (1995); Whitelam et al., Biochem. Soc. Trans. 22:940-944 (1994); and references cited therein. See, also generally for plant expression of antibodies, but not limited to, Each of the above references is entirely incorporated herein by reference.

The antibodies of the invention can bind human RSV with a wide range of affinities ( $K_D$ ). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human RSV with high affinity. For example, a human mAb can bind human RSV with a  $K_D$  equal to or less than about  $10^{-7}$  M, such as but not limited to, 0.1-9.9 (or any range or value therein)  $\times 10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$ ,  $10^{-10}$ ,  $10^{-11}$ ,  $10^{-12}$ ,  $10^{-13}$  or any range or value therein.

The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, *et al.*, "Antibody-Antigen Interactions," In

*Fundamental Immunology*, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis *Immunology*, W. H. Freeman and Company: New York, NY (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g.,  $K_D$ ,  $K_a$ ,  $K_d$ ) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein.

#### Nucleic Acid Molecules

Using the information provided herein, such as the nucleotide sequences encoding at least 70-100% of the contiguous amino acids of at least one of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one RSV antibody can be obtained using methods described herein or as known in the art.

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited to, at least one specified portion of at least one CDR, as CDR1, CDR2 and/or CDR3 of at least one heavy chain or light chain; nucleic acid molecules comprising the coding sequence for an RSV antibody or variable region (e.g., any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F); and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one RSV antibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific RSV antibodies of the present invention. See, e.g., Ausubel, et al., *supra*, and such nucleic acid variants are included in the present invention. Non-limiting examples of isolated nucleic acid molecules of the present invention include the CDR sequences of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, corresponding to

non-limiting examples of a nucleic acid encoding, respectively, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, LC CDR3, HC variable region and LC variable region.

As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an RSV antibody can include, but are not limited to, those encoding the amino acid sequence of an antibody fragment, by itself; the coding sequence for the entire antibody or a portion thereof; the coding sequence for an antibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an antibody can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused antibody comprising an antibody fragment or portion.

#### **Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein**

The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode at least a portion of an antibody encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic

acid sequences that can be employed for selective hybridization to a polynucleotide encoding an antibody of the present invention. See, e.g., Ausubel, *supra*; Colligan, *supra*, each entirely incorporated herein by reference.

#### **Construction of Nucleic Acids**

5           The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

          The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable  
10       sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the coding sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

15           Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*)

#### **Recombinant Methods for Constructing Nucleic Acids**

20           The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and  
25       construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*)

#### **Nucleic Acid Screening and Isolation Methods**

          A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention, such as those disclosed herein. Probes can be used to hybridize  
30       with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity



between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Patent No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*.)

For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, *supra*, Sambrook, *supra*, and Ausubel, *supra*, as well as Mullis, et al., U.S. Patent No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

### Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization  
5 with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

### Recombinant Expression Cassettes

10 The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding an antibody of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention  
15 operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous  
20 form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* or *in vitro* by mutation, deletion and/or substitution.

### Vectors And Host Cells

The present invention also relates to vectors that include isolated nucleic acid molecules of  
25 the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one RSV antibody by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium  
30 phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed

region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

5        Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, US Pat.Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017, ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase (GS, US Pat.Nos. 5,122,464; 5,770,359; 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for  
10        culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or  
15        other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

At least one antibody of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be  
20        added to the N-terminus of an antibody to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to an antibody of the present invention to facilitate purification. Such regions can be removed prior to final preparation of an antibody or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74;  
25        Ausubel, supra, Chapters 16, 17 and 18.

Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.

Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding an antibody of the present  
30        invention. Such methods are well known in the art, e.g., as described in US patent Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

Illustrative of cell cultures useful for the production of the antibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers

of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7  
5 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va (www.atcc.org). Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly  
10 preferred embodiment, the recombinant cell is a P3X63Ab8.653 or a SP2/0-Ag14 cell.

Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (US Pat.Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (US Pat.No. 5,266,491), at least one  
15 human immunoglobulin promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and  
20 Hybridomas (www.atcc.org) or other known or commercial sources.

When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J.  
25 Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

### **Purification of an Antibody**

An RSV antibody can be recovered and purified from recombinant cell cultures by well-  
30 known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can



also be employed for purification. See, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

Antibodies of the present invention include naturally purified products, products of chemical  
5 synthetic procedures, and products produced by recombinant techniques from a eukaryotic host,  
including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host  
employed in a recombinant production procedure, the antibody of the present invention can be  
glycosylated or can be non-glycosylated, with glycosylated preferred. Such methods are described in  
many standard laboratory manuals, such as Sambrook, supra, Sections 17.37-17.42; Ausubel, supra,  
10 Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12-14, all entirely  
incorporated herein by reference.

### **RSV Proteins and Antibodies**

The isolated proteins and antibodies of the present invention comprise at least one protein  
15 and/or antibody amino acid sequence disclosed or described herein encoded by any suitable  
polynucleotide, or any at least one isolated or prepared protein antibody. Preferably, the at least one  
protein has at least one RSV activity and the at least one antibody binds human RSV and, thereby  
partially or substantially modulates at least one structural or biological activity of at least one RSV  
protein.

20 As used herein, the term "RSV protein" refers to a protein as described herein that has at least  
one RSV-dependent activity, such as 5-10000%, of the activity of a known or other RSV protein or  
active portion thereof, preferably by at least about 10, 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91,  
92, 93, 94, 95, 96, 97, 98, 99, 100% or more, depending on the assay. The capacity of a RSV protein to  
have at least one RSV-dependent activity is preferably assessed by at least one suitable RSV protein or  
25 receptor assay, as described herein and/or as known in the art.

As used herein, the term "neutralizing antibody" refers to an antibody that can inhibit at least  
one RSV-dependent activity by about 5-120%, preferably by at least about 10, 20, 30, 40, 50, 55, 60, 65,  
70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or more depending on the assay. The  
capacity of an RSV antibody to inhibit an RSV-dependent activity is preferably assessed by at least one  
30 suitable RSV protein or receptor assay, as described herein and/or as known in the art. An antibody of  
the invention can be of any class (IgG, IgA, IgM, IgE, IgD, etc.) or isotype and can comprise a kappa or  
lambda light chain. In one embodiment, the human antibody comprises an IgG heavy chain or defined

fragment, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. Antibodies of this type can be prepared by employing a transgenic mouse or other transgenic non-human mammal comprising at least one human light chain (e.g., IgG, IgA and IgM (e.g.,  $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$ ,  $\gamma 4$ ) transgenes as described herein and/or as known in the art. In another embodiment, the human RSV human antibody comprises an IgG1 heavy chain and a IgG1 light chain.

At least one antibody of the invention binds at least one specified epitope specific to at least one RSV protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of the protein, which epitope can optionally comprise at least one portion of at least one extracellular, soluble, hydrophilic, external or cytoplasmic portion of the protein. The at least one specified epitope can comprise any combination of at least one amino acid sequence of at least 1-3 amino acids to the entire specified portion of contiguous amino acids of any RSV protein, such as the F glycoprotein.

The at least one antibody of the present invention can preferably comprise at least one antigen-binding region that comprises at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one heavy chain variable region and/or at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one light chain variable region. As a non-limiting example, the antibody can comprise at least one of the heavy chain CDRs of at least one SEQ ID NOS:1, 2 or 3; at least one light chain CDR of at least one of SEQ ID NOS:4,5 and/or 6. In a particular embodiment, the protein and antibody can have an antigen-binding region that comprises at least a portion of at least one heavy chain (HC) CDR (i.e., HC CDR1, HC CDR2 and/or HC CDR3) having the amino acid sequence of the corresponding HC CDRs 1, 2 and/or 3. In another particular embodiment, the antibody or antigen-binding portion or variant can have at least one antigen-binding region that comprises at least a portion of at least one light chain (LC) CDR (i.e., LC CDR1, LC CDR2 and/or LC CDR3). In a preferred embodiment the three heavy chain CDRs and the three light chain CDRs of the antibody or antigen-binding fragment have the amino acid sequence of the corresponding CDR of at least one of mAb H1L1, H1L2, H2L1, H2L2, H1L3, H2L3, , , as described herein. Such antibodies can be prepared by chemically joining together the various portions (e.g., CDRs, framework) of the antibody using conventional techniques, by preparing and expressing a (i.e., one or more) nucleic acid molecule that encodes the antibody using conventional techniques of recombinant DNA technology or by using any other suitable method.

The RSV antibody can comprise at least one of a heavy or light chain variable region having a defined amino acid sequence. For example, in a preferred embodiment, the RSV antibody

comprises at least one of at least one heavy chain variable region, optionally having the amino acid sequence of at least one of SEQ ID NOS:7, 8 and/or 9; and/or at least one light chain variable region, optionally having the amino acid sequence of at least one of SEQ ID NOS:9, 10 and/or 11.

Antibodies that bind to human RSV and that comprise a defined heavy or light chain variable region can be prepared using suitable methods, such as phage display (Katsube, Y., *et al.*, *Int J Mol. Med*, 1(5):863-868 (1998)) or methods that employ transgenic animals, as known in the art and/or as described herein. For example, a transgenic mouse, comprising a functionally rearranged human immunoglobulin heavy chain transgene and a transgene comprising DNA from a human immunoglobulin light chain locus that can undergo functional rearrangement, can be immunized with human RSV or a fragment thereof to elicit the production of antibodies. If desired, the antibody producing cells can be isolated and hybridomas or other immortalized antibody-producing cells can be prepared as described herein and/or as known in the art. Alternatively, the antibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.

The invention also relates to antibodies, antigen-binding fragments, immunoglobulin chains and CDRs comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such antibodies or antigen-binding fragments and antibodies comprising such chains or CDRs can bind human RSV with high affinity (e.g.,  $K_D$  less than or equal to about  $10^{-9}$  M). Amino acid sequences that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g, charge, structure, polarity, hydrophobicity/ hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

#### Amino Acid Codes

The amino acids that make up RSV antibodies of the present invention are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., *et al.*, *Molecular Biology of The Cell*, Third Ed., Garland Publishing, Inc., New York, 1994):

SINGLE LETTER CODE	THREE LETTER CODE	NAME	THREE NUCLEOTIDE CODON(S)
A	Ala	Alanine	GCA, GCC, GCG, GCU
C	Cys	Cysteine	UGC, UGU
D	Asp	Aspartic acid	GAC, GAU
E	Glu	Glutamic acid	GAA, GAG
F	Phe	Phenylalanine	UUC, UUU
G	Gly	Glycine	GGA, GGC, GGG, GGU
H	His	Histidine	CAC, CAU
I	Ile	Isoleucine	AUA, AUC, AUU
K	Lys	Lysine	AAA, AAG
L	Leu	Leucine	UUA, UUG, CUA, CUC, CUG, CUU
M	Met	Methionine	AUG
N	Asn	Asparagine	AAC, AAU
P	Pro	Proline	CCA, CCC, CCG, CCU
Q	Gln	Glutamine	CAA, CAG
R	Arg	Arginine	AGA, AGG, CGA, CGC, CGG, CGU
S	Ser	Serine	AGC, AGU, UCA, UCC, UCG, UCU
T	Thr	Threonine	ACA, ACC, ACG, ACU
V	Val	Valine	GUA, GUC, GUG, GUU
W	Trp	Tryptophan	UGG
Y	Tyr	Tyrosine	UAC, UAU

An RSV antibody of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

5 Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for any given RSV antibody, fragment or variant will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, such as 1-30 or any range or value therein, as specified herein.

10 Amino acids in an RSV antibody of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to at  
15 least one RSV neutralizing activity. Sites that are critical for antibody binding can also be identified



by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

RSV proteins of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from 3-100 to all of the contiguous amino acids of at least one of any known RSV F protein. RSV antibodies of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from 5 to all of the contiguous amino acids of at least one of any 5-500 amino acid portion of SEQ ID NOS:1-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, preferably and optionally including at least one of the corresponding CDRs.

Non-limiting variants that can enhance or maintain at least one of the listed activities include, but are not limited to, any of the above polypeptides, further comprising at least one mutation corresponding to at least one substitution selected from the group consisting of 1-50 amino acids of of at least one of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F.

A(n) RSV antibody can further optionally comprise a polypeptide of at least one of 70-100% of the contiguous amino acids of at least one of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, or any variant thereof.

In one embodiment, the amino acid sequence of a RSV protein or antibody has about 70-100% identity (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the amino acid sequence of the corresponding chain of at least one of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F. Preferably, 70-100% amino acid identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art.

Exemplary heavy chain and light chain variable regions sequences are provided in SEQ ID NOS: 7-12. The proteins and antibodies of the present invention, or specified variants thereof, can comprise any number of contiguous amino acid residues from an antibody of the present invention, wherein that number is selected from the group of integers consisting of from 10-100% of the number of contiguous residues in an RSV protein or antibody. Optionally, this subsequence of contiguous amino acids is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino acids in length, or any range or value therein. Further, the

number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes at least one biologically active protein or antibody of the present invention. Biologically active proteins or antibodies have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-1000% of that of the native (non-synthetic), endogenous or related and known protein or antibody. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

In another aspect, the invention relates to RSV proteins or antibodies of the invention, as described herein, which are modified by the covalent attachment of a moiety. Such modification can produce a RSV protein or antibody with improved pharmacokinetic properties (e.g., increased *in vivo* serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

The modified proteins and antibodies of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody or protein. Each organic moiety that is bonded to the protein or antibody of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, a RSV antibody or protein modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying antibodies or proteins of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrrolidone. Preferably, the hydrophilic polymer that modifies the protein or antibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example PEG<sub>5000</sub> and PEG<sub>20,000</sub>, wherein the subscript is the average molecular weight of

the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N, N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying antibodies of the invention include, for example, n-dodecanoate (C<sub>12</sub>, laurate), n-tetradecanoate (C<sub>14</sub>, myristate), n-octadecanoate (C<sub>18</sub>, stearate), n-eicosanoate (C<sub>20</sub>, arachidate), n-docosanoate (C<sub>22</sub>, behenate), n-triacontanoate (C<sub>30</sub>), n-tetracontanoate (C<sub>40</sub>), *cis*- $\Delta^9$ -octadecanoate (C<sub>18</sub>, oleate), all *cis*- $\Delta^{5,8,11,14}$ -eicosatetraenoate (C<sub>20</sub>, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

The modified human proteins and antibodies can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C<sub>1</sub>-C<sub>12</sub> group wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, -(CH<sub>2</sub>)<sub>3</sub>-, -NH-(CH<sub>2</sub>)<sub>6</sub>-NH-, -(CH<sub>2</sub>)<sub>2</sub>-NH- and -CH<sub>2</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-

CH-NH-. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, *et al.*, WO 92/16221 the entire teachings of which are incorporated herein by reference.)

Modified proteins or antibodies of the invention can be produced by reacting the protein or antibody with a modifying agent. For example, the organic moieties can be bonded to the antibody or protein in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified RSV proteins or antibodies can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of the protein and antibody. The reduced protein and antibody can then be reacted with a thiol-reactive modifying agent to produce the modified antibody of the invention. Modified proteins and antibodies comprising an organic moiety that is bonded to specific sites of an antibody of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch *et al.*, *Bioconjugate Chem.*, 3:147-153 (1992); Werlen *et al.*, *Bioconjugate Chem.*, 5:411-417 (1994); Kumaran *et al.*, *Protein Sci.* 6(10):2233-2241 (1997); Itoh *et al.*, *Bioorg. Chem.*, 24(1): 59-68 (1996); Capellas *et al.*, *Biotechnol. Bioeng.*, 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996).

#### IDIOTYPE ANTIBODIES TO RSV ANTIBODY COMPOSITIONS

In addition to monoclonal or chimeric RSV antibodies, the present invention is also directed to an idiotypic (Id) antibody specific for such antibodies of the invention. An anti-Id antibody is an antibody that recognizes unique determinants generally associated with the antigen-binding region of another antibody. The Id can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the Id antibody with the antibody or a CDR containing region thereof. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-Id antibody.



**RSV PROTEIN AND ANTIBODY COMPOSITIONS**

The present invention also provides at least one RSV antibody or protein composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more RSV antibodies or proteins thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring compositions comprising at least one or two RSV antibody or protein amino acid sequences selected from the group consisting of 5-100% of the contiguous amino acids of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, or specified fragments, domains or variants thereof. Preferred RSV antibody compositions include at least one or two full length, fragments, domains or variants as at least one CDR containing portions of the RSV antibody sequence of 70-100% of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, or specified fragments, domains or variants thereof. Further preferred compositions comprise 40-99% of at least one of 70-100% of any 5-500 amino acid portion of SEQ ID NOS:7-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, or specified fragments, domains or variants thereof. Such composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein.

RSV antibody or protein compositions of the present invention can further comprise at least one of any suitable and effective amount of a composition or pharmaceutical composition comprising at least one RSV antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy, optionally further comprising at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluroquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization,

an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Non-limiting examples of such cytokines include, but are not limited to, any of IL-1 to IL-23. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2<sup>nd</sup> Edition, Appleton and Lange, Stamford, CT (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

Such compositions can also include toxin molecules that are associated, bound, co-formulated or co-administered with at least one antibody or protein of the present invention. The toxin can optionally act to selectively kill the pathologic cell or tissue. The pathologic cell can be a cancer or other cell. Such toxins can be, but are not limited to, purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin, e.g., selected from at least one of ricin, diphtheria toxin, a venom toxin, or a bacterial toxin. The term toxin also includes both endotoxins and exotoxins produced by any naturally occurring, mutant or recombinant bacteria or viruses which may cause any pathological condition in humans and other mammals, including toxin shock, which can result in death. Such toxins may include, but are not limited to, enterotoxigenic *E. coli* heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), *Shigella* cytotoxin, *Aeromonas* enterotoxins, toxic shock syndrome toxin-1 (TSST-1), Staphylococcal enterotoxin A (SEA), B (SEB), or C (SEC), Streptococcal enterotoxins and the like. Such bacteria include, but are not limited to, strains of a species of enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (e.g., strains of serotype O157:H7), Staphylococcus species (e.g., *Staphylococcus aureus*, *Staphylococcus pyogenes*), *Shigella* species (e.g., *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*), *Salmonella* species (e.g., *Salmonella typhi*, *Salmonella cholera-suis*, *Salmonella enteritidis*), *Clostridium* species (e.g., *Clostridium perfringens*, *Clostridium difficile*, *Clostridium botulinum*), *Camphlobacter* species (e.g., *Camphlobacter jejuni*, *Camphlobacter fetus*), *Heliobacter* species, (e.g., *Heliobacter pylori*), *Aeromonas* species (e.g., *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas caviae*), *Pleisomonas shigelloides*, *Yersina enterocolitica*, *Vibrios* species (e.g., *Vibrios cholerae*, *Vibrios parahemolyticus*), *Klebsiella* species, *Pseudomonas aeruginosa*, and *Streptococci*. See, e.g., Stein, ed., INTERNAL MEDICINE, 3rd ed., pp 1-13, Little, Brown and Co., Boston,

(1990); Evans et al., eds., *Bacterial Infections of Humans: Epidemiology and Control*, 2d. Ed., pp 239-254, Plenum Medical Book Co., New York (1991); Mandell et al, *Principles and Practice of Infectious Diseases*, 3d. Ed., Churchill Livingstone, New York (1990); Berkow et al, eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Wood et al, *FEMS Microbiology Immunology*, 76:121-134 (1991); Marrack et al, *Science*, 248:705-711 (1990), the contents of which references are incorporated entirely herein by reference.

RSV antibody or protein compounds, compositions or combinations of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like.

10 Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the RSV antibody or protein composition as well known

15 in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present

20 singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary but non-limiting protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine,

25 methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol,

30 xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

RSV antibody or protein compositions can also include a buffer or a pH adjusting agent;

typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

5        Additionally, RSV antibody or protein compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl- $\beta$ -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g.,  
10        cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the RSV antibody or protein compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19<sup>th</sup> ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52<sup>nd</sup> ed., Medical Economics, Montvale, NJ (1998), the  
15        disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

### Formulations

As noted above, the invention provides for stable formulations, which is preferably a  
20        phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one RSV antibody or protein in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl  
25        alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03,  
30        0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2%



m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

5           As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one RSV antibody or protein with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further  
10       comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one RSV antibody or protein, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one RSV antibody or protein in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

15           The at least one RSV antibody or protein used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

          The range of at least one RSV antibody in at least one product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0  
20       ng/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

          The range of at least one RSV antibody in at least one product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0  
25       μg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

          Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and  
30       the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an microbial effect. Such concentrations are dependent on the preservative

selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyols, other block copolymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

The formulations of the present invention can be prepared by a process which comprises mixing at least one RSV antibody or protein and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one RSV antibody or protein and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one RSV antibody or protein in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one RSV antibody or protein that is reconstituted with a

second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

5           The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40°C and retain the biological activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution  
10       can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

          The solutions of at least one RSV antibody or protein in the invention can be prepared by a process that comprises mixing at least one antibody or protein in an aqueous diluent.  
15       Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one antibody or protein in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the  
20       temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

          The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one RSV antibody or protein that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring  
25       reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

          The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one RSV antibody or protein that is reconstituted with a second vial  
30       containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one antibody or protein solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as BD Pens, BD Autojector<sup>®</sup>, Humaject<sup>®</sup>, NovoPen<sup>®</sup>, B-D<sup>®</sup>Pen, AutoPen<sup>®</sup>, and OptiPen<sup>®</sup>, GenotropinPen<sup>®</sup>, Genotronorm Pen<sup>®</sup>, Humatro Pen<sup>®</sup>, Reco-Pen<sup>®</sup>, Roferon Pen<sup>®</sup>, Biojector<sup>®</sup>, iject<sup>®</sup>, J-tip Needle-Free Injector<sup>®</sup>, Intraject<sup>®</sup>, Medi-Ject<sup>®</sup>, e.g., as made or  
5 developed by Becton Dickenson (Franklin Lakes, NJ, [www.bectondickenson.com](http://www.bectondickenson.com)), Disetronic (Burgdorf, Switzerland, [www.disetronic.com](http://www.disetronic.com); Bioject, Portland, Oregon ([www.bioject.com](http://www.bioject.com)); National Medical Products, Weston Medical (Peterborough, UK, [www.weston-medical.com](http://www.weston-medical.com)), Medi-Ject Corp (Minneapolis, MN, [www.mediject.com](http://www.mediject.com)). Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for  
10 delivery of the reconstituted solution such as the HumatroPen<sup>®</sup>.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one RSV antibody or protein in the aqueous diluent to form a  
15 solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The formulations of the present invention can be prepared by a process that  
20 comprises mixing at least one RSV antibody or protein and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one antibody or protein and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one antibody or protein in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the  
25 protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed stable or preserved formulations can be provided to patients as clear  
30 solutions or as dual vials comprising a vial of lyophilized at least one RSV antibody or protein that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more



convenient treatment regimen than currently available.

At least one RSV antibody or protein in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary,  
5 transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

#### Therapeutic Applications

The present invention also provides a method for modulating or treating at least one RSV related disease, in a cell, tissue, organ, animal, or patient, as known in the art or as described  
10 herein, using at least one antibody or protein of the present invention.

The present invention also provides a method for modulating or treating at least one adult or pediatric RSV related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, lower respiratory infections, pneumonia, tracheobronchitis, bronchiolitis, bronchitis, and any related infections or inflammatory disorders, such as but not limited to at least one of, or at least one  
15 inflammation related to, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, adult respiratory distress syndrome, allergic rhinitis, perennial rhinitis, asthma, systemic anaphalaxis, receptor hypersensitivity reactions, chronic obstructive pulmonary disease (COPD), hypersensitivity pneumonitis, granulomas due to intracellular organisms, drug sensitivity,  
20 cachexia, cystic fibrosis, neonatal chronic lung disease; at least one infectious disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections, HIV infection, HIV neuropathy, meningitis, hepatitis (A,B or C, or the like), septic arthritis, peritonitis, pneumonia, epiglottitis, e. coli 0157:h7, hemolytic uremic syndrome,  
25 thrombolytic thrombocytopenic purpura, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, mycobacterium tuberculosis, mycobacterium avium intracellulare, pneumocystis carinii pneumonia, pelvic inflammatory disease, orchitis, epididymitis, legionella, lyme disease, influenza a, epstein-barr virus, vital-associated hemaphagocytic syndrome, vital encephalitis, aseptic meningitis, and the like. Such a method can  
30 optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one RSV antibody or protein to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one RSV antibody or protein to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such diseases, wherein the administering of said at least one RSV antibody or protein, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2<sup>nd</sup> Edition, Appleton and Lange, Stamford, CT (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

TNF antagonists suitable for compositions, combination therapy, co-administration, devices and/or methods of the present invention (further comprising at least one anti body, specified portion and variant thereof, of the present invention), include, but are not limited to, TNF antibodies, antigen-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide,

tenidap, phosphodiesterase inhibitors (e.g, pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds  
5 which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e.g., captopril); and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors.

As used herein, a "tumor necrosis factor antibody," "TNF antibody," "TNF $\alpha$  antibody," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNF $\alpha$  activity *in vitro*,  
10 *in situ* and/or preferably *in vivo*. For example, a suitable TNF human antibody of the present invention can bind TNF $\alpha$  and includes TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNF $\alpha$ . A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF  
15 production and/or synthesis.

Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse human TNF $\alpha$  IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody.  
20 The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNF $\alpha$  in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant  
25 human TNF $\alpha$ , the affinity constant of chimeric antibody cA2 was calculated to be  $1.04 \times 10^{10} \text{M}^{-1}$ . Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, *et al.*, *antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988; Colligan *et al.*, eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992-2000); Kozbor *et al.*, *Immunol. Today*, 4:72-79 (1983); Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987-2000); and Muller, *Meth. Enzymol.*, 92:589-601 (1983), which references are entirely incorporated herein by reference.

In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line

designated c134A. Chimeric antibody cA2 is produced by a cell line designated c168A.

Additional examples of monoclonal TNF antibodies that can be used in the present invention are described in the art (see, e.g., U.S. Patent No. 5,231,024; Möller, A. *et al.*, *Cytokine* 2(3):162-169 (1990); U.S. Application No. 07/943,852 (filed September 11, 1992); Rathjen *et al.*, International  
5 Publication No. WO 91/02078 (published February 21, 1991); Rubin *et al.*, EPO Patent Publication No. 0 218 868 (published April 22, 1987); Yone *et al.*, EPO Patent Publication No. 0 288 088 (October 26, 1988); Liang, *et al.*, *Biochem. Biophys. Res. Comm.* 137:847-854 (1986); Meager, *et al.*, *Hybridoma* 6:305-311 (1987); Fendly *et al.*, *Hybridoma* 6:359-369 (1987); Bringman, *et al.*, *Hybridoma* 6:489-507 (1987); and Hirai, *et al.*, *J. Immunol. Meth.* 96:57-62 (1987), which references  
10 are entirely incorporated herein by reference).

### **TNF Receptor Molecules**

Preferred TNF receptor molecules useful in the present invention are those that bind TNF $\alpha$  with high affinity (see, e.g., Feldmann *et al.*, International Publication No. WO 92/07076 (published April 30, 1992); Schall *et al.*, *Cell* 61:361-370 (1990); and Loetscher *et al.*, *Cell* 61:351-359 (1990),  
15 which references are entirely incorporated herein by reference) and optionally possess low immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran *et al.*, *Eur. J. Biochem.* 223:831-840 (1994)), are also useful in the present invention. Truncated forms  
20 of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNF $\alpha$  inhibitory binding proteins (Engelmann, H. *et al.*, *J. Biol. Chem.* 265:1531-1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be  
25 used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, can contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide  
30 linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. Application No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein



by reference.

TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer *et al.*, *Eur. J. Immunol.* 21:2883-2886 (1991); Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Peppel *et al.*, *J. Exp. Med.* 174:1483-1489 (1991); Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Butler *et al.*, *Cytokine* 6(6):616-623 (1994); Baker *et al.*, *Eur. J. Immunol.* 24:2040-2048 (1994); Beutler *et al.*, U.S. Patent No. 5,447,851; and U.S. Application No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon *et al.*, U.S. Patent No. 5,116,964; Capon *et al.*, U.S. Patent No. 5,225,538; and Capon *et al.*, *Nature* 337:525-531 (1989), which references are entirely incorporated herein by reference.

A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF $\alpha$  with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF $\alpha$  with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience, New York (1987-2000).

Cytokines include any known cytokine. See, e.g., CopewithCytokines.com. Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

**Therapeutic Treatments.** Any method of the present invention can comprise a method for

treating a RSV mediated disorder or disease, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one RSV antibody or protein to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such disorders or diseases, wherein the administering of said at least one RSV antibody or protein, further comprises administering, before concurrently, and/or after, at least one selected from at least one at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluroquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist.

## **Protein Dosing**

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one RSV protein composition that total, on average, a range from at least about 0.001 ng to 500 milligrams of at least one RSV protein per kilogram of patient per dose, and preferably from at least about 0.1 ng to 100 milligrams antibody /kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.0001ng –0.05 mg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the

particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

5 Preferred doses of at least one protein can optionally include 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and/or 100-500 micrograms or  
10 milligrams/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14, 14.5, 15, 15.5, 15.9, 16, 16.5, 16.9, 17, 17.5,  
15 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 ng or  $\mu\text{g/ml}$  serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the  
20 pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1  $\mu\text{g}$  to 100 milligrams per kilogram of body weight. Ordinarily 0.0001 to 50, and preferably 0.001 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain  
25 desired results.

As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one antibody of the present invention 0.1 to 100  $\mu\text{g/kg}$ , such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000 or 3000  
30  $\mu\text{g/kg}$ , per day, or 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or

additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated  
5 doses.

Dosage forms (composition) suitable for internal administration generally contain from about 0.00001 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

10 Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one RSV antibody composition that total, on average, a range from at least about 0.00001 to 500 milligrams of at least one RSV antibody per kilogram of patient per dose, and preferably from at least about 0.0001 to 100 milligrams antibody /kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum  
15 concentration can comprise 0.0001-500 µg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a particular monitored  
20 or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

#### **Antibody Dosing**

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one RSV antibody composition that total, on average, a range from at least about  
25 0.001 ng to 500 milligrams of at least one RSV antibody per kilogram of patient per dose, and preferably from at least about 0.1 ng to 100 milligrams antibody /kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.0001ng –0.05 mg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend  
30 upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a



particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

Preferred doses of at least one antibody can optionally include 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and/or 100-500 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 20, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14, 14.5, 15, 15.5, 15.9, 16, 16.5, 16.9, 17, 17.5, 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 µg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one antibody of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated doses.

Dosage forms (composition) suitable for internal administration generally contain from about

0.1 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

#### **Administration**

5 For parenteral administration, the antibody or protein can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that  
10 maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

#### **Alternative Administration**

15 Many known and developed modes of can be used according to the present invention for administering pharmaceutically effective amounts of at least one RSV antibody according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

RSV antibodies of the present invention can be delivered in a carrier, as a solution, emulsion,  
20 colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

#### **Parenteral Formulations and Administration**

Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated  
25 naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile  
30 involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection

device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

#### **Alternative Delivery**

The invention further relates to the administration of at least one RSV antibody by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. At least one RSV antibody composition can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) or any other administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as, but not limited to, creams and suppositories; for buccal, or sublingual administration such as, but not limited to, in the form of tablets or capsules; or intranasally such as, but not limited to, the form of powders, nasal drops or aerosols or certain agents; or transdermally such as not limited to a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

#### **Pulmonary/Nasal Administration**

For pulmonary administration, preferably at least one RSV antibody composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one RSV antibody can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of antibodies are also known in the art.

All such devices can use of formulations suitable for the administration for the dispensing of antibody in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin<sup>®</sup> metered dose inhaler, typically use a propellant gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 5 98/35888). Dry powder inhalers like Turbuhaler<sup>™</sup> (Astra), Rotahaler<sup>®</sup> (Glaxo), Diskus<sup>®</sup> (Glaxo), Spiros<sup>™</sup> inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler<sup>®</sup> powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, US 5458135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx<sup>™</sup> Aradigm, the Ultravent<sup>®</sup> nebulizer 10 (Mallinckrodt), and the Acorn II<sup>®</sup> nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as 15 limiting the scope of the invention. Preferably, a composition comprising at least one RSV antibody is delivered by a dry powder inhaler or a sprayer. There are a several desirable features of an inhalation device for administering at least one antibody of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10  $\mu\text{m}$ , preferably 20 about 1-5  $\mu\text{m}$ , for good respirability.

#### **Administration of RSV antibody Compositions as a Spray**

A spray including RSV antibody composition can be produced by forcing a suspension or solution of at least one RSV antibody through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired 25 output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one RSV antibody composition delivered by a sprayer have a particle size less than about 10  $\mu\text{m}$ , preferably in the range of about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

Formulations of at least one RSV protein or antibody composition suitable for use with a 30 sprayer typically include antibody or protein compositions in an aqueous solution at a concentration of about 0.000001 mg to about 1000 mg of at least one RSV antibody or protein composition per ml of solution or mg/gm, or any range or value therein, e.g., but not limited to, .1, .2, .3, .4, .5, .6, .7, .8, .9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,



30, 40, 45, 50, 60, 70, 80, 90 or 100 ng or  $\mu\text{g}$  or mg/ml or ng or  $\mu\text{g}$  or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the antibody composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody compositions include sucrose, mannitol, lactose, trehalose, glucose, or the like. The antibody composition formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the antibody or protein composition caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as RSV antibodies, or specified portions or variants, can also be included in the formulation.

#### **Administration of RSV antibody compositions by a Nebulizer**

antibody composition can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of antibody composition through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition either directly or through a coupling fluid, creating an aerosol including the antibody composition. Advantageously, particles of antibody composition delivered by a nebulizer have a particle size less than about 10  $\mu\text{m}$ , preferably in the range of about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

Formulations of at least one RSV antibody suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one RSV antibody protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can

also include an excipient or agent for stabilization of the at least one RSV antibody composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one RSV antibody compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one RSV antibody include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one RSV antibody formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one RSV antibody caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as antibody protein can also be included in the formulation.

#### **Administration of RSV antibody compositions By A Metered Dose Inhaler**

In a metered dose inhaler (MDI), a propellant, at least one RSV antibody, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10  $\mu\text{m}$ , preferably about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ . The desired aerosol particle size can be obtained by employing a formulation of antibody composition produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one RSV antibody for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one RSV antibody as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one RSV antibody as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for

formulation of a protein such as protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one RSV antibody compositions via devices not described herein.

## 5 Oral Formulations and Administration

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and 10 trasyolol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive 15 diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution 20 preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. 25 No. 5,587,753 are used to deliver biologically active agents orally are known in the art.

## Mucosal Formulations and Administration

For absorption through mucosal surfaces, compositions and methods of administering at least one RSV antibody include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which 30 promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g.

suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like (U.S. Pat. Nos. 5,849,695).

### **Transdermal Formulations and Administration**

For transdermal administration, the at least one RSV antibody is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. Nos. 5,814,599).

### **Prolonged Administration and Formulations**

It can be sometimes desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in



animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

- 5           Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

**Example 1: Expression and Purification of a RSV Protein or Antibody in *E. coli***

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., Chatsworth, CA). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and  
5 contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a protein or antibody can be inserted in such a way as to produce that protein or antibody with the six His  
10 residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that protein or antibody. However, a protein or antibody coding sequence can optionally be inserted such that translation of the six His codons is prevented and, therefore, a protein or antibody is produced with no 6 X His tag.

The nucleic acid sequence encoding the desired portion of a RSV protein or antibody lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR  
15 oligonucleotide primers (based on the sequences presented, e.g., as presented in at least one of SEQ ID NOS:7-12, or any portion thereof or nucleic acid encoding thereof, or any portion of Figs 2-5 , which anneal to the amino terminal encoding DNA sequences of the desired portion of a RSV protein or antibody and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added  
20 to the 5' and 3' sequences, respectively.

For cloning a RSV protein or antibody, the 5' and 3' primers have nucleotides corresponding or complementary to a portion of the coding sequence of a RSV, e.g., as presented in at least one of SEQ ID NOS:1-12, or any portion thereof or nucleic acid encoding thereof, or any portion of Figs 2-5, according to known method steps. One of ordinary skill in the art would appreciate, of course, that  
25 the point in a protein or antibody coding sequence where the 5' primer begins can be varied to amplify a desired portion of the complete protein or antibody shorter or longer than the mature form.

The amplified RSV nucleic acid fragments and the vector pQE60 are digested with appropriate restriction enzymes and the digested DNAs are then ligated together. Insertion of the RSV DNA into the restricted pQE60 vector places a RSV protein or antibody coding region  
30 including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG codon. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures

such as those described in Sambrook, et al., 1989; Ausubel, 1987-1998. *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing RSV protein or antibody, is available commercially from QIAGEN, Inc. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the RSV is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, a protein or antibody can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors.

If insoluble protein is generated, the protein is made soluble according to known method steps. After renaturation the protein or antibody is purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column is used to obtain pure RSV protein or antibody. The purified protein or antibody is stored at 4°C or frozen at -40°C to -120°C.

## **Example 2: Cloning and Expression of a RSV Polypeptide in a Baculovirus Expression System**

In this illustrative example, the plasmid shuttle vector pA2 GP is used to insert the cloned DNA encoding the mature protein or antibody into a baculovirus to express a RSV protein or antibody, using a baculovirus leader and standard methods as described in Summers, et al., A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong

polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 protein or antibody and convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid  
5 contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Other baculovirus vectors are used in place of the vector above, such as pAc373, pVL941 and  
10 pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow, et al., *Virology* 170:31-39.

The cDNA sequence encoding the mature RSV protein or antibody in the deposited or other  
15 clone, lacking the AUG initiation codon and the naturally associated nucleotide binding site, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or complementary to a portion of the coding sequence of a RSV protein or antibody, e.g., as presented in at least one of SEQ ID NOS:1-12, or any portion thereof or nucleic acid encoding thereof, or any portion of Figs 2-  
20 5, according to known method steps.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit (e.g., "Geneclean," BIO 101 Inc., La Jolla, CA). The fragment then is then digested with the appropriate restriction enzyme and again is purified on a 1% agarose gel. This fragment is designated herein "F1".

25 The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, CA). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase.  
30 *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human RSV gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so



that only those bacterial colonies containing the RSV gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac RSV.

Five  $\mu$ g of the plasmid pBacRSV is co-transfected with 1.0  $\mu$ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner, et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987).

1  $\mu$ g of BaculoGold™ virus DNA and 5  $\mu$ g of the plasmid pBac RSV are mixed in a sterile well of a microtiter plate containing 50  $\mu$ l of serum-free Grace's medium (Life Technologies, Inc., Rockville, MD). Afterwards, 10  $\mu$ l Lipofectin plus 90  $\mu$ l Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, according to known methods. An agarose gel with "Blue Gal" (Life Technologies, Inc., Rockville, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies, Inc., Rockville, MD, page 9-10). After appropriate incubation, blue stained plaques are picked with a micropipettor tip (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-RSV.

To verify the expression of the RSV gene, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-RSV at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available, e.g., from Life Technologies, Inc., Rockville, MD). If radiolabeled protein or antibodies are desired, 42 hours later, 5 mCi of 35S-methionine and 5 mCi 35S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The protein or

antibodies in the supernatant as well as the intracellular protein or antibodies are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified protein or antibody can be used to determine the amino terminal sequence of the mature protein or antibody and thus the cleavage point and length of the secretory signal peptide.

### **Example 3: Cloning and Expression of RSV protein or antibody in Mammalian Cells**

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the antibody coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clontech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human HeLa 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein or antibody, e.g., as a desired portion of at least one of any 5-500 amino acid portion of SEQ ID NOS:1-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., Biochem. J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are

used for the production of antibodies or proteins of the present invention.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., *Molec. Cell. Biol.* 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

#### Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of RSV antibody or protein, e.g., using a coding sequence for at least one of any 5-500 amino acid portion of SEQ ID NOS:1-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., *J. Biol. Chem.* 253:1357-1370 (1978); J. L. Hamlin and C. Ma, *Biochem. et Biophys. Acta* 1097:101-113 (1990); and M. J. Page and M. A. Sydenham, *Biotechnology* 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains coding DNA for expressing the gene of interest (e.g., encoding at least one of SEQ IDN NOS:1-12) under control of the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., *Molec. Cell. Biol.* 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., *Cell* 41:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40

early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the RSV in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It can be advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the desired RSV antibody or protein is used, e.g., DNA or RNA coding for at least one of any 5-500 amino acid portion of SEQ ID NOS:1-12, any portion of Figures 3-4 or encoded by any portion of Figures 2A-G or 5A-F, corresponding to at least one portion of at least one RSV antibody protein of the present invention, according to known method steps.

The isolated encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection.  $\mu\text{g}$  of the expression plasmid pC4 is cotransfected with 0.5  $\mu\text{g}$  of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1  $\mu\text{g}$  /ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1  $\mu\text{g}$  /ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

#### **Example 2: Generation of Antibodies Reactive With Human RSV Using Transgenic Mice**



## Summary

Transgenic mice have been used that contain human heavy and light chain immunoglobulin genes to generate high affinity, completely human, monoclonal antibodies that can be used therapeutically to inhibit the action of RSV for the treatment of one or more RSV-mediated disease.

5 (CBA/J x C57/BL6/J) F<sub>2</sub> hybrid mice containing human variable and constant region antibody transgenes for both heavy and light chains are immunized with human recombinant RSV (Taylor et al., Intl. Immunol. 6:579-591 (1993); Lonberg, et al., Nature 368:856-859 (1994); Neuberger, M., Nature Biotech. 14:826 (1996); Fishwild, et al., Nature Biotechnology 14:845-851 (1996)). Several fusions yield one or more panels of completely human RSV reactive IgG monoclonal antibodies. The

10 completely human RSV antibodies are further characterized. All are IgG1κ. Such antibodies are found to have affinity constants somewhere between  $1 \times 10^9$  and  $9 \times 10^{12}$ . The high affinities of these fully human monoclonal antibodies make them suitable candidates for therapeutic applications in RSV related diseases, pathologies or disorders.

## Abbreviations

- 15 BSA - bovine serum albumin
- CO<sub>2</sub> - carbon dioxide
- DMSO - dimethyl sulfoxide
- EIA - enzyme immunoassay
- FBS - fetal bovine serum
- 20 H<sub>2</sub>O<sub>2</sub> - hydrogen peroxide
- HRP - horseradish peroxidase\
- ID - interadermal
- Ig - immunoglobulin
- RSV - respiratory syncytial virus
- 25 IP - intraperitoneal
- IV - intravenous
- Mab - monoclonal antibody
- OD - optical density
- OPD - o-Phenylenediamine dihydrochloride
- 30 PEG - polyethylene glycol
- PSA - penicillin, streptomycin, amphotericin
- RT - room temperature
- SQ - subcutaneous
- v/v - volume per volume
- 35 w/v - weight per volume

## Materials and Methods

### Animals

Transgenic mice that can express human antibodies are known in the art (and are

40 commercially available (e.g., from GenPharm International, San Jose, CA; Abgenix, Fremont, CA,

and others) that express human immunoglobulins but not mouse IgM or Igκ. For example, such transgenic mice contain human sequence transgenes that undergo *V(D)J* joining, heavy-chain class switching, and somatic mutation to generate a repertoire of human sequence immunoglobulins (Lonberg, et al., Nature 368:856-859 (1994)). The light chain transgene can be derived, e.g., in part from a yeast artificial chromosome clone that includes nearly half of the germline human Vκ region. In addition, the heavy-chain transgene can encode both human μ and human γ1 (Fishwild, et al., Nature Biotechnology 14:845-851 (1996)) and/or γ3 constant regions. Mice derived from appropriate genotypic lineages can be used in the immunization and fusion processes to generate fully human monoclonal antibodies to RSV.

## 10 Immunization

One or more immunization schedules using at least one RSV protein as an immunogen as generated according to known methods (e.g., as provided in Example 1) can be used to generate the RSV human hybridomas. The first several fusions can be performed after the following exemplary immunization protocol, but other similar known protocols can be used. Several 14-20 week old female and/or surgically castrated transgenic male mice are immunized IP and/or ID with 1-1000 μg of recombinant human RSV protein emulsified with an equal volume of TITERMAX or complete Freund's adjuvant in a final volume of 100-400 μL (e.g., 200). Each mouse can also optionally receive 1-10 μg in 100 μL physiological saline at each of 2 SQ sites. The mice can then be immunized 1-7, 5-12, 10-18, 17-25 and/or 21-34 days later IP (1-400 μg) and SQ (1-400 μg x 2) with RSV emulsified with an equal volume of TITERMAX or incomplete Freund's adjuvant. Mice can be bled 12-25 and 25-40 days later by retro-orbital puncture without coagulant. The blood is then allowed to clot at RT for one hour and the serum is collected and titered using an RSV EIA assay according to known methods. Fusions are performed when repeated injections do not cause titers to increase. At that time, the mice can be given a final IV booster injection of 1-400 μg RSV diluted in 100 μL physiological saline. Three days later, the mice can be euthanized by cervical dislocation and the spleens removed aseptically and immersed in 10 mL of cold phosphate buffered saline (PBS) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (PSA). The splenocytes are harvested by sterilely perfusing the spleen with PSA-PBS. The cells are washed once in cold PSA-PBS, counted using Trypan blue dye exclusion and resuspended in RPMI 1640 media containing 25 mM Hepes.

## Cell Fusion

Fusion can be carried out at a 1:1 to 1:10 ratio of murine myeloma cells to viable spleen cells according to known methods, e.g., as known in the art. As a non-limiting example, spleen cells and

myeloma cells can be pelleted together. The pellet can then be slowly resuspended, over 30 seconds, in 1 mL of 50% (w/v) PEG/PBS solution (PEG molecular weight 1,450, Sigma) at 37 °C. The fusion can then be stopped by slowly adding 10.5 mL of RPMI 1640 medium containing 25 mM Hepes (37 °C) over 1 minute. The fused cells are centrifuged for 5 minutes at 500-1500 rpm. The cells are then resuspended in HAT medium (RPMI 1640 medium containing 25 mM Hepes, 10% Fetal Clone I serum (Hyclone), 1 mM sodium pyruvate, 4 mM L-glutamine, 10 µg/mL gentamicin, 2.5% Origen culturing supplement (Fisher), 10% 653-conditioned RPMI 1640/Hepes media, 50 µM 2-mercaptoethanol, 100 µM hypoxanthine, 0.4 µM aminopterin, and 16 µM thymidine) and then plated at 200 µL/well in fifteen 96-well flat bottom tissue culture plates. The plates are then placed in a humidified 37 °C incubator containing 5% CO<sub>2</sub> and 95% air for 7-10 days.

#### **Detection of Human IgG RSV antibodies in Mouse Serum**

Solid phase EIA's can be used to screen mouse sera for human IgG antibodies specific for human RSV protein. Briefly, plates can be coated with RSV protein at 2 µg/mL in PBS overnight. After washing in 0.15M saline containing 0.02% (v/v) Tween 20, the wells can be blocked with 1% (w/v) BSA in PBS, 200 µL/well for 1 hour at RT. Plates are used immediately or frozen at -20 °C for future use. Mouse serum dilutions are incubated on the RSV coated plates at 50 µL/well at RT for 1 hour. The plates are washed and then probed with 50 µL/well HRP-labeled goat human IgG, Fc specific diluted 1:30,000 in 1% BSA-PBS for 1 hour at RT. The plates can again be washed and 100 µL/well of the citrate-phosphate substrate solution (0.1M citric acid and 0.2M sodium phosphate, 0.01% H<sub>2</sub>O<sub>2</sub> and 1 mg/mL OPD) is added for 15 minutes at RT. Stop solution (4N sulfuric acid) is then added at 25 µL/well and the OD's are read at 490 nm via an automated plate spectrophotometer.

#### **Detection of Completely Human Immunoglobulins in Hybridoma Supernates**

Growth positive hybridomas secreting fully human immunoglobulins can be detected using a suitable EIA. Briefly, 96 well pop-out plates (VWR, 610744) can be coated with 10 µg/mL goat human IgG Fc in sodium carbonate buffer overnight at 4 °C. The plates are washed and blocked with 1% BSA-PBS for one hour at 37°C and used immediately or frozen at -20 °C. Undiluted hybridoma supernatants are incubated on the plates for one hour at 37°C. The plates are washed and probed with HRP labeled goat human kappa diluted 1:10,000 in 1% BSA-PBS for one hour at 37°C. The plates are then incubated with substrate solution as described above.

#### **Determination of Fully Human RSV Reactivity**

Hybridomas, as above, can be simultaneously assayed for reactivity to RSV using a suitable RIA or other assay. For example, supernatants are incubated on goat human IgG Fc plates as above, washed and then probed with radiolabeled RSV with appropriate counts per well for 1 hour at RT.

The wells are washed twice with PBS and bound radiolabeled RSV is quantitated using a suitable counter.

Human IgG1 $\kappa$  RSV secreting hybridomas can be expanded in cell culture and serially subcloned by limiting dilution. The resulting clonal populations can be expanded and cryopreserved in freezing medium (95% FBS, 5% DMSO) and stored in liquid nitrogen.

#### Isotyping

Isotype determination of the antibodies can be accomplished using an EIA in a format similar to that used to screen the mouse immune sera for specific titers. RSV protein can be coated on 96-well plates as described above and purified antibody at 2  $\mu$ g/mL can be incubated on the plate for one hour at RT. The plate is washed and probed with HRP labeled goat human IgG<sub>1</sub> or HRP labeled goat human IgG<sub>3</sub> diluted at 1:4000 in 1% BSA-PBS for one hour at RT. The plate is again washed and incubated with substrate solution as described above.

#### Binding Kinetics of Human Human RSV antibodies With Human RSV

Binding characteristics for antibodies can be suitably assessed using an RSV capture EIA and BIAcore technology, for example. Graded concentrations of purified human RSV antibodies can be assessed for binding to EIA plates coated with 2  $\mu$ g/mL of RSV in assays as described above. The OD's can be then presented as semi-log plots showing relative binding efficiencies.

Quantitative binding constants can be obtained, e.g., as follows, or by any other known suitable method. A BIAcore CM-5 (carboxymethyl) chip is placed in a BIAcore 2000 unit. HBS buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v P20 surfactant, pH 7.4) is flowed over a flow cell of the chip at 5  $\mu$ L/minute until a stable baseline is obtained. A solution (100  $\mu$ L) of 15 mg of EDC (N-ethyl-N'-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride) in 200  $\mu$ L water is added to 100  $\mu$ L of a solution of 2.3 mg of NHS (N-hydroxysuccinimide) in 200  $\mu$ L water. Forty (40)  $\mu$ L of the resulting solution is injected onto the chip. Six  $\mu$ L of a solution of human RSV (15  $\mu$ g/mL in 10 mM sodium acetate, pH 4.8) is injected onto the chip, resulting in an increase of ca. 500 RU. The buffer is changed to TBS/Ca/Mg/BSA running buffer (20 mM Tris, 0.15 M sodium chloride, 2 mM calcium chloride, 2 mM magnesium acetate, 0.5% Triton X-100, 25  $\mu$ g/mL BSA, pH 7.4) and flowed over the chip overnight to equilibrate it and to hydrolyze or cap any unreacted succinimide esters.

Antibodies are dissolved in the running buffer at 33.33, 16.67, 8.33, and 4.17 nM. The flow rate is adjusted to 30  $\mu$ L/min and the instrument temperature to 25  $^{\circ}$ C. Two flow cells are used for the kinetic runs, one on which RSV protein had been immobilized (sample) and a second, underivatized flow cell (blank). 120  $\mu$ L of each antibody concentration is injected over the flow cells



at 30  $\mu\text{L}/\text{min}$  (association phase) followed by an uninterrupted 360 seconds of buffer flow (dissociation phase). The surface of the chip is regenerated (respiratory syncytial virus /antibody complex dissociated) by two sequential injections of 30  $\mu\text{L}$  each of 2 M guanidine thiocyanate.

Analysis of the data is done using BIA evaluation 3.0 or CLAMP 2.0, as known in the art.

- 5 For each antibody concentration the blank sensogram is subtracted from the sample sensogram. A global fit is done for both dissociation ( $k_d, \text{sec}^{-1}$ ) and association ( $k_a, \text{mol}^{-1} \text{sec}^{-1}$ ) and the dissociation constant ( $K_D, \text{mol}$ ) calculated ( $k_d/k_a$ ). Where the antibody affinity is high enough that the RUs of antibody captured are  $>100$ , additional dilutions of the antibody are run.

## Results and Discussion

### 10 Generation of Human RSV Monoclonal antibodies

Several fusions are performed and each fusion is seeded in 15 plates (1440 wells/fusion) that yield several dozen antibodies specific for human RSV protein. Of these, some are found to consist of a combination of human and mouse Ig chains. The remaining hybridomas secrete RSV antibodies consisting solely of human heavy and light chains. Of the human hybridomas all are expected to be

15 IgG1 $\kappa$ .

### Binding Kinetics of Human Human RSV antibodies

- ELISA analysis confirms that purified antibody from most or all of these hybridomas bind RSV protein in a concentration-dependent manner. Figures 1-2 show the results of the relative binding efficiency of these antibodies. In this case, the avidity of the antibody for its cognate antigen
- 20 (epitope) is measured. It should be noted that binding RSV directly to the EIA plate can cause denaturation of the protein and the apparent binding affinities cannot be reflective of binding to undenatured protein. Fifty percent binding is found over a range of concentrations.

- Quantitative binding constants are obtained using BIAcore analysis of the human antibodies and reveals that several of the human monoclonal antibodies are very high affinity with  $K_D$  in the
- 25 range of  $1 \times 10^{-8}$  to  $7 \times 10^{-12}$ .

## Conclusions

- Several fusions are performed utilizing splenocytes from hybrid mice containing human variable and constant region antibody transgenes that are immunized with human RSV. A set of several completely human RSV reactive IgG monoclonal antibodies of the IgG1 $\kappa$  isotype are
- 30 generated. The completely human RSV antibodies are further characterized. Several of generated antibodies have affinity constants between  $1 \times 10^8$  and  $9 \times 10^{12}$ . The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable for therapeutic applications in RSV-dependent diseases, pathologies or related conditions.

It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the  
5 above teachings and, therefore, are within the scope of the appended claims.

CLAIMS

We claim:

- 5 1 . At least one isolated mammalian RSV antibody, comprising at least one variable region comprising at least one heavy chain and at least one light chain of SEQ ID NOS:7-12.
- 10 2 . At least one isolated mammalian RSV antibody, comprising either (i) at least two of the heavy chain complementarity determining regions (CDR) amino acid sequences of at least one of SEQ ID NOS:1-3; or (ii) at least two of the light chain CDR amino acids sequences of at least one of SEQ ID NOS:4-6.
- 15 3 . At least one isolated mammalian RSV antibody, comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1-6.
- 4 . At least one isolated mammalian RSV antibody that binds to the same region of a RSV polypeptide as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.
- 20 5 . At least one isolated mammalian RSV antibody, comprising at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO:7-12.
- 6 . An RSV antibody according to any of claims 1-5, wherein said antibody binds RSV with an affinity of at least one selected from at least  $10^{-9}$  M, at least  $10^{-10}$  M, at least  $10^{-11}$  M, or at least  $10^{-12}$  M.
- 25 7 . An RSV antibody according to to any of claims 1-6, wherein said antibody substantially modulates at least one activity of at least one RSV polypeptide.
- 8 . An isolated nucleic acid encoding at least one isolated mammalian RSV antibody according to any of claims 1-6 and having at least one human CDR of SEQ ID NOS:1-6.
- 30 9 . An isolated nucleic acid vector comprising an isolated nucleic acid according to claim 9.
- 10 . A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to claim 9.
- 11 . A host cell according to claim 10, wherein said host cell is at least one

selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.

12 . A method for producing at least one RSV antibody, comprising translating a nucleic acid according to claim 9 under conditions in vitro, in vivo or in situ, such that the RSV antibody is expressed in detectable or recoverable amounts.

13 . A composition comprising at least one isolated mammalian RSV antibody according to any of claims 1-6 having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9, and at least one pharmaceutically acceptable carrier or diluent.

14 . A composition according to claim 13, further comprising at least one at least one compound or polypeptide selected from at least one of a detectable label or reporter, a TNF antagonist, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, or a cytokine antagonist.

15 . An anti-idiotypic antibody or fragment that specifically binds at least one RSV antibody according to any of claims 1-6.

16 . A method for diagnosing or treating a RSV related condition in a cell, tissue, organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one antibody according to any of claims 1-6, with, or to, said cell, tissue, organ or animal.

17 . A method according to claim 16, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

18 . A method according to claim 16, wherein said contacting or said administering is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelical, intracerebellar, intracerebroventricular, intracolical, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.



19 .           A method according to 16, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or polypeptide selected from at least one of a detectable label or reporter, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, or a cytokine antagonist.

20 .           A medical device, comprising at least one RSV antibody according to any of claims 1-6, wherein said device is suitable to contacting or administering said at least one RSV antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

21 .           An article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one RSV antibody according to any of claims 1-6.

22 .           The article of manufacture of claim 21, wherein said container is a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

23 .           A method for producing at least one isolated mammalian RSV antibody according to any of claims 1-6, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody. At least one RSV antibody produced by a method according to claim 23.

HC -

HCH1

EVQLLEESGGGLVRLPGSLRLSCAASGTTLSGYTMHWVRQAPGKGLEWVSSITGGSNFINYSDSVKG  
RFTISRDNAKNSLYLQMNSLTAE~~DTAVYYCATAPIAPPYFDHWGQGT~~LVTVSS

HCH2

EVQLLEESGGGLVRLPGSLRLSCAASGTTLSGYTMHWVRQAPGKGLEWVSSITGGSNFINYSDSVKGR  
FTISRDNAKNSLYLQMNSLTAE~~DTAVYYCATAPIAPPYFDHWGQGT~~LVTVSS

LC -

L1

DIQMTQSPSSLSASVGDRVTITCRATOSVSNFLN~~WYQQKPGEAP~~TLLIYDASTSOSGVPSRFSGSGS  
GMDFSLTIS~~SLQPEDLAMYYCOASINTPL~~FGGGTRIDMR

L2

DIQMTQSPSSLSASVGDRVTITCRATOSVSNFLN~~WYQQKPGEAP~~TLLIYDASTSOSGVPSRFSGSGS  
GMDFSLTIS~~SLQPEDLAMYYCOASINTPL~~FGGGTRVDIK

L3

DAELTQSPSSLSASVGDRVTITCRATOSVSNFLN~~WYQQKPGEAP~~TLLIYDASTSOSGVPSRFSGSGS  
GMDFSLTIS~~SLQPEDLAMYYCOASINTPL~~FGGGTRIDMR

L4

AELTQSPSSLSASVGDRVTITCRATOSVSNFLN~~WYQQKPGEAP~~TLLIYDASTSOSGVPSR  
FSGSGSGMDFSLTIS~~SLQPEDLAMYYCOASINTPL~~FGGGTRIDMR

FIGURE 1

Variable Region

RSV HC vers1  
RSV HC vers2

GAGGTGCAGCTGCTCGACGAGTCTGGGGGAGGGCGCTGCTCAGGCTGGGGGTCCCTAAGACTC  
GAGGTGCAGCTGCTCGACGAGTCTGGGGGAGGGCGCTGCTCAGGCTGGGGGTCCCTAAGACTC

Variable Region

RSV HC vers1  
RSV HC vers2

TCGTGTGCAGCTGCTCGACGAGTCTGGGGGAGGGCGCTGCTCAGGCTGGGGGTCCCTAAGACTC  
TCGTGTGCAGCTGCTCGACGAGTCTGGGGGAGGGCGCTGCTCAGGCTGGGGGTCCCTAAGACTC

Variable Region

RSV HC vers1  
RSV HC vers2

GTAGCAACTTCATATAAAGTACTACTCAGACTGAGTCAAGGCG  
GTAGCAACTTCATATAAAGTACTACTCAGACTGAGTCAAGGCG

Variable Region

RSV HC vers1  
RSV HC vers2

AGCCGAGGACACCGGCTGCTATTAATTCGCG  
AGCCGAGGACACCGGCTGCTATTAATTCGCG

FIGURE 2

Variable Region

RSV HC vers1	EVQLLEISGGGEVFRPGGSLRLISCAASGTTLS	GYTHF	WVRQAPGKGLEWVS	SI	TGGSNF	INYS	SDSVKGRFTI	SRD	NAKNSLY
RSV HC vers2	EVQLLEISGGGEVFRPGGSLRLISCAASGTTLS	GYTHF	WVRQAPGKGLEWVS	SI	TGGSNF	INYS	SDSVKGRFTI	SRD	NAKNSLY

Variable Region

RSV HC vers1	LQMNSLTAEDTAVYYCAT	APTAPPYFDE	WGQGLVTVSS
RSV HC vers2	LQMNSLTAEDTAVYYCAT	APTAPPYFDE	WGQGLVTVSS

FIGURE 3



Variable Region

	CDR1		CDR2	
RSV LC vers1	DQMTQSPSSLSASVGDRVTITC	RATQSVSNFLN	WYQQKPGEAPTLLTY	DASTSQS
RSV LC vers2	DQMTQSPSSLSASVGDRVTITC	RATQSVSNFLN	WYQQKPGEAPTLLTY	DASTSQS
RSV LC vers3	DAELTQSPSSLSASVGDRVTITC	RATQSVSNFLN	WYQQKPGEAPTLLTY	DASTSQS
RSV LC vers4	-AEETQSPSSLSASVGDRVTITC	RATQSVSNFLN	WYQQKPGEAPTLLTY	DASTSQS

Variable Region

	CDR3	
RSV LC vers1	SLTISSLOPEDLAMYYC	QASINTPE
RSV LC vers2	SLTISSLOPEDLAMYYC	QASINTPE
RSV LC vers3	SLTISSLOPEDLAMYYC	QASINTPE
RSV LC vers4	SLTISSLOPEDLAMYYC	QASINTPE

FIGURE 4

Variable Region

RSV LC vers1  
RSV LC vers2  
RSV LC vers3  
RSV LC vers4

GATATCCAGATGACGGCAGTGTCCATTCCTGGCTGTCTGCATCTGTAGGAGAC  
GATATCCAGATGACGGCAGTGTCCATTCCTGGCTGTCTGCATCTGTAGGAGAC  
GATGCGGAGCTGAGCCAGTGTCCATTCCTGGCTGTCTGCATCTGTAGGAGAC  
G---CCGAGCTGACCCGAGTGTCCATTCCTGGCTGTCTGCATCTGTAGGAGAC

Variable Region

RSV LC vers1  
RSV LC vers2  
RSV LC vers3  
RSV LC vers4

	CDR1	CDR2
RSV LC vers1	AGAGTCACCATCCTTGG	CEGGCAACTCAGAGTGTAGTAACTTTTAAAT
RSV LC vers2	AGAGTCACCATCCTTGG	CEGGCAACTCAGAGTGTAGTAACTTTTAAAT
RSV LC vers3	AGAGTCACCATCCTTGG	CEGGCAACTCAGAGTGTAGTAACTTTTAAAT
RSV LC vers4	AGAGTCACCATCCTTGG	CEGGCAACTCAGAGTGTAGTAACTTTTAAAT

Variable Region

RSV LC vers1  
RSV LC vers2  
RSV LC vers3  
RSV LC vers4

	CDR2
RSV LC vers1	CATCCACTTCCGAAAGT
RSV LC vers2	CATCCACTTCCGAAAGT
RSV LC vers3	CATCCACTTCCGAAAGT
RSV LC vers4	CATCCACTTCCGAAAGT

Variable Region

RSV LC vers1  
RSV LC vers2  
RSV LC vers3  
RSV LC vers4

	CDR3
RSV LC vers1	AAATGTATTACTGT
RSV LC vers2	AAATGTATTACTGT
RSV LC vers3	AAATGTATTACTGT
RSV LC vers4	AAATGTATTACTGT

FIGURE 5

## SEQUENCE LISTING

<110> Centocor Inc.  
Scallan, Bernard J.

<120> RSV PROTEINS, ANTIBODIES, COMPOSITIONS, METHODS AND USES

<130> CEN0203

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Val Ser Ser Ile Thr Gly Gly Ser Asn Phe Ile Asn Tyr Ser Asp Ser  
 50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu  
 65 70 75 80



Tyr Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Ala Val Tyr Tyr  
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Tyr Asp Ala Ser Thr Ser Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
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 65 70 75 80

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